

Claims

1. A method for identifying and/or detecting T-cell epitopes of a protein antigen in vitro, where a population of peptide fragments of the antigen is subjected to competitive binding to a first immobilized receptor unit, preferably in the presence of a second receptor unit which, together with the first receptor unit, is capable of forming a receptor, where at least one peptide fragment with affinity to the receptor binds to at least the first, preferably both, receptor unit(s), and the bound peptide fragment is then isolated and analyzed, comprising

- a) immobilization of at least the first receptor unit which has at least one first functional group on a nanoparticle, the surface of which has at least one second functional group which binds the first functional group,
- b) preparation of a population of peptide fragments of the protein antigen which comprises different sequence ranges of the protein antigen,
- c) carrying out a competitive binding of the peptide fragment population to the first receptor unit immobilized on the nanoparticle, preferably in the presence of a second receptor unit, where at least one peptide fragment having affinity to at least the first receptor unit, preferably to both

receptor units, if appropriate together with the second receptor unit, binds to the first receptor unit, giving a receptor/peptide fragment complex immobilized on the nanoparticle, and

- d) analysis of the immobilized receptor/peptide fragment complex and/or the bound peptide fragment.

2. The method as claimed in claim 1, where prior to the competitive binding reaction the second receptor unit is present free in solution.

3. The method as claimed in claim 1, where prior to the competitive binding reaction the second receptor unit is, together with the first receptor unit, immobilized on the nanoparticle, in the form of a dimer which forms the receptor.

4. The method as claimed in claim 3, where the second receptor unit has at least one third functional group and the surface of the nanoparticle has at least one fourth functional group which binds the third functional group.

5. The method as claimed in claim 3 or 4, where the receptor is immobilized on the nanoparticle in a targeted manner and with conservation of its biological activity.

6. The method as claimed in any of claims 1 to 5, where the receptor is a major histocompatibility complex (MHC) molecule, the receptor/peptide fragment complex is a peptide-presenting MHC molecule and the first and the second receptor unit are chains of the MHC molecule.

7. The method as claimed in claim 6, where the receptor is an MHC molecule of class I.

8. The method as claimed in claim 6 or 7, where the first receptor unit is a heavy chain of about 45 kDa and the second receptor unit is a light chain of about 12 kDa or the first receptor unit is a light chain of about 12 kDa and the second receptor unit is a heavy chain of about 45 kDa.

9. The method as claimed in any of claims 6 to 8, where the heavy chain is an HLA-A, HLA-B or HLA-C monomer and the light chain is β -2-microglobulin.

10. The method as claimed in any of claims 6 to 9, where the peptide fragment bound in the peptide-presenting MHC molecule originates from an endogenous protein antigen.

11. The method as claimed in any of claims 6 to 10, where the peptide fragment bound in the receptor/peptide fragment complex comprises about 8 to 10 amino acids.

12. The method as claimed in claim 6, where the receptor is an MHC molecule of class II.

13. The method as claimed in claim 12, where the first receptor unit is an α -chain of about 34 kD and the second receptor unit is a β -chain of about 30 kD or the first receptor unit is a β -chain of about 30 kDa and the second receptor unit is an α -chain of about 34 kDa.

14. The method as claimed in claim 12 or 13, where the α -chain and the β -chain are HLA-DR, HLA-DQ or HLA-DP monomers.

15. The method as claimed in any of claims 12 to 14, where the peptide fragment bound in the receptor/peptide fragment complex originates from an exogenous protein antigen.

16. The method as claimed in any of claims 12 to 15, where the peptide fragment bound in the receptor/peptide fragment complex comprises about 15 to 24 amino acids.

17. The method as claimed in any of claims 1 to 16, where the first and the second receptor unit are natural chains or chains prepared by genetic engineering or chemical synthesis.

18. The method as claimed in claim 17, where the first functional group is a natural component of the first receptor unit or is introduced into the first receptor unit by genetic engineering, biochemical, enzymatical and/or chemical derivatization or chemical synthesis.

19. The method as claimed in claim 17 or 18, where the third functional group is a natural component of the second receptor unit or is introduced into the second receptor unit by genetic engineering, biochemical, enzymatical and/or chemical derivatization or chemical synthesis.

20. The method as claimed in claim 18 or 19, where the first functional group and the third functional group are different from one another and are selected from the group consisting of carboxyl groups, amino groups, thiol groups, biotin groups, His tag, FLAG tag, Strep tag I groups, Strep tag II groups, histidine tag groups and FLAG tag groups.

21. The method as claimed in any of claims 1 to 20, where the second functional group on the surface of the nanoparticle, which binds the first functional group, and the fourth functional group on the surface of the nanoparticle, which binds the third functional group, are different from one another and selected from the group consisting of amino groups, carboxyl groups, maleinimido groups, avidin groups, streptavidin groups, neutravidin groups and metal chelate complexes.

22. The method as claimed in claim 21, where the second functional group and the fourth functional group are applied to the surface of the nanoparticle using graft silanization, silanization, chemical derivatization and similar suitable methods.

23. The method as claimed in claim 21 or 22, where the nanoparticles have a core of chemically inert material, preferably silicon dioxide.

24. The method as claimed in claim 23, where the nanoparticles have a diameter of 30 to 400 nm, preferably 50 to 150 nm.

25. The method as claimed in any of claims 1 to 24, where the population of peptide fragments of the protein antigen is prepared by enzymatic protein cleavage, genetic engineering or chemical synthesis.

26. The method as claimed in claim 25, where the peptide fragments of the population completely represent the entire

amino acid sequence of the protein antigen.

27. The method as claimed in claim 25, where the peptide fragments of the population only partially represent the amino acid sequence of the protein antigen.

28. The method as claimed in claim 27, where the peptide fragments of the population have amino acid sequences which represent the predicted potential T-cell epitopes.

29. The method as claimed in claim 27 or 28, where the predicted potential T-cell epitopes are determined using a computer algorithm.

30. The method as claimed in any of claims 25 to 29, where the peptide fragments have a length of 8 to 10 amino acids if the receptor is an MHC molecule type I.

31. The method as claimed in any of claims 25 to 29, where the peptide fragments have a length of 15 to 24 amino acids if the receptor is an MHC molecule type II.

32. The method as claimed in any of claims 25 to 31, where the peptide fragments of the population are, prior to the competitive binding, provided with a marker and/or a fifth functional group.

33. The method as claimed in claim 32, where the marker is a fluorescent marker or a radioactive marker.

34. The method as claimed in claim 32, where the fifth functional group is different from the first, second, third and/or fourth functional group and does not bond to these.

35. The method as claimed in any of claims 1 to 34, where the immobilization of the first receptor unit or the immobilization of the first and second receptor unit on the nanoparticles is carried out by incubating the receptor unit(s) with the nanoparticles in a PBS buffer for a period of 1 h to 4 h, preferably 2 h, at room temperature in a shaking apparatus, affording nanoparticles having immobilized first receptor units or nanoparticles having immobilized first and second receptor units.

36. The method as claimed in any of claims 1 to 34, where the immobilization of receptor unit(s) on the nanoparticles is carried out by preparing in solution a receptor/peptide complex using a peptide of known sequence and suitable length, the first receptor unit and the second receptor unit, immobilizing the receptor/peptide complex on the nanoparticles, subjecting the nanoparticles having the immobilized receptor/peptide complex to a treatment to remove at least the bound peptide, giving nanoparticles having immobilized receptor units.

37. The method as claimed in claim 36, where the receptor/peptide complex is prepared by incubation of the first receptor unit, the second receptor unit and the peptide in a buffer comprising 100 mM Tris, 2 mM EDTA, 400 mM L-arginine, 5 mM reduced glutathione and 0.5 mM oxidized glutathione for a period of more than 36 h, preferably 48 h, at a temperature of below 20°C, preferably 10°C.

38. The method as claimed in claim 36 or 37, where the receptor/peptide complex is immobilized on the nanoparticles only by binding of the first functional group of the first receptor unit to the second functional group of the nanoparticles.

39. The method as claimed in claim 36 or 37, where the receptor/peptide complex is immobilized on the nanoparticles by binding of the first functional group of the first receptor unit to the second functional group of the nanoparticles and by binding of the third functional group of the second receptor unit to the fourth functional group of the nanoparticles.

40. The method as claimed in any of claims 36 to 39, where the nanoparticles which have the immobilized receptor/peptide complex are treated with a stripping buffer, pH 3.0, which comprises 50 mM sodium citrate, for a period of less than 20 s, preferably 10 s, to remove the bound peptide.

41. The method as claimed in claim 40, where, if the receptor/peptide complex is immobilized only by binding of the first functional group to the second functional group of the nanoparticles, in the treatment of the nanoparticles obtained in addition to the peptide the second receptor unit, too, is removed from the nanoparticles, giving nanoparticles having the immobilized first receptor unit.

42. The method as claimed in claim 40, where, if the receptor/peptide complex is immobilized on the nanoparticles

by binding of the first functional group of the first receptor unit to the second functional group of the nanoparticles and binding of the third functional group of the second receptor unit to the fourth functional group of the nanoparticles, in the treatment of the nanoparticles obtained, only the bound peptide is removed from the nanoparticles, giving nanoparticles having the immobilized first and second receptor units.

43. The method as claimed in any of claims 35 to 42, where the nanoparticles having the immobilized receptor unit(s) are removed from the buffer by at least one centrifugation and at least one washing and are resuspended in a buffer.

44. The method as claimed in any of claims 35 to 43, where the competitive binding of the peptide fragment population on the nanoparticles which have the first or the first and second immobilized receptor unit is carried out by incubation of the peptide fragment population with the nanoparticles in a PBS buffer over a period of 2 h to 6 h, preferably 4 h, at a temperature of from room temperature to 39°C, preferably 37°C.

45. The method as claimed in claim 44, where the PBS buffer contains the second receptor unit if the nanoparticles have only the immobilized first receptor unit.

46. The method as claimed in claim 44 or 45, where the nanoparticles, after binding of the peptide fragment with the

highest affinity to the two receptor units with formation of an immobilized receptor/peptide fragment complex are removed from the buffer and the unbound peptide fragments by at least one centrifugation and at least one washing and are resuspended in a buffer.

47. The method as claimed in any of claims 1 to 46, where the suspension of the nanoparticles having the immobilized receptor/peptide fragment complex with the bound peptide fragment is analyzed using matrix-assisted laser desorption/ionization (MALDI) methods, in particular the MALDI-TOF (time-of-flight) method.

48. The method as claimed in claim 47, where the nanoparticle suspension obtained after centrifugation and washing is deposited on a MALDI sample stage and analyzed.

49. The method as claimed in claim 47 or 48, where the matrix used during the MALDI method is applied before or after deposition of the nanoparticle-containing suspension or jointly therewith on the MALDI sample stage.

50. The method as claimed in any of claims 1 to 46, where the peptide fragment bound in the immobilized receptor/peptide fragment complex is removed from the complex by dissolution, isolated and analyzed.

51. The method as claimed in claim 50, where the nanoparticles having the immobilized receptor/peptide fragment complex are treated with a stripping buffer, pH 3.0, which comprises 50 mM of sodium citrate, for a period of less

than 20 s, preferably 10 s, and the peptide fragment goes into solution.

52. The method as claimed in claim 50 or 51, where the nanoparticles are removed by centrifugation from the solution which contains the peptide fragment.

53. The method as claimed in any of claims 50 to 52, where the solution which contains the released peptide fragment is brought into contact with nanoparticles which have six functional groups which bind the fifth functional group of the peptide fragment, so that the peptide fragment is immobilized on the nanoparticles by binding of the fifth functional group to the sixth functional group, and the nanoparticles which have the immobilized peptide fragment are removed from the solution.

54. The method as claimed in any of claims 50 to 53, where the immobilized peptide fragment is removed from the nanoparticles and sequenced.

55. A method for identifying and/or preparing a peptide vaccine against a protein antigen, where the amino acid sequence of a T-cell epitope of the protein antigen is identified in vitro, a peptide having the identified amino acid sequence is prepared and a peptide-presenting major histocompatibility complex (MHC) is prepared using the prepared peptide and a first and second chain, which method comprises

- a) providing a population of peptide fragments of the protein antigen,
- b) providing nanoparticles having, at their surface, at least one first immobilized chain of an MHC molecule, where the chain has a conformation which allows formation of an MHC molecule,
- c) carrying out competitive binding of the peptide fragment population to the first chain immobilized on the nanoparticles in the presence of a second chain of an MHC molecule, where the peptide fragment having the greatest affinity to the two chains of the MHC molecule binds together with the second chain to the first chain, giving a peptide fragment-presenting MHC molecule, and
- d) isolation of the peptide fragment from the MHC molecule to identify a peptide fragment suitable for a peptide vaccine, and determination of its amino acid sequence, and optional practice of steps e) to h), namely
- e) preparation, by genetic engineering or chemical synthesis, of suitable amounts of a peptide based on the determined amino acid sequence of the peptide fragment,
- f) preparation, by genetic engineering or chemical synthesis, of suitable amounts of the first and second chains,

- g) preparation of suitable amounts of peptide-presenting MHC molecules by joint incubation of the first chain, the second chain and the peptide prepared, and
- h) preparation of a peptide vaccine in the form of a lyophilizate or an aqueous colloidal solution or suspension of the peptide-presenting MHC molecules.

56. The method as claimed in claim 55, where in addition to the first chain, the second chain, too, is immobilized on the surface of the nanoparticles.

57. The method as claimed in claim 55 or 56, where the two chains are immobilized on the surface of the nanoparticle in the form of a dimer which forms the MHC molecule.

58. The method as claimed in any of claims 55 to 57, where the population of peptide fragments of the protein antigen is prepared by enzymatic protein cleavage, genetic engineering or chemical synthesis.

59. The method as claimed in claim 58, where the peptide fragments of the population completely represent the entire amino acid sequence of the protein antigen.

60. The method as claimed in claim 58, where the peptide fragments of the population only partially represent the amino acid sequence of the protein antigen.

61. The method as claimed in claim 60, where the peptide fragments of the population have amino acid sequences which represent potential T-cell epitopes determined using a

computer algorithm.

62. The method as claimed in any of claims 58 to 61, where the peptide fragments or the peptide have/has a length of 8 to 10 amino acids if the peptide fragment or peptide-presenting MHC molecule to be prepared is an MHC molecule type I, or a length of 15 to 24 amino acids if the peptide fragment or peptide-presenting MHC molecule to be prepared is an MHC molecule type II.

63. The method as claimed in any of claims 55 to 62, where the first chain is a heavy chain of about 45 kD, the second chain is a light chain of about 12 kD and the two chains are capable of forming an MHC molecule type I.

64. The method as claimed in claim 63, where the first chain is an HLA-A, HLA-B or HLA-C monomer and the second chain is β -2-microglobulin.

65. The method as claimed in any of claims 55 to 62, where the first chain is an α -chain of about 34 kD, the second chain is a β -chain of about 30 kD and the two chains are capable of forming an MHC molecule type II.

66. The method as claimed in claim 65, where the first chain and the second chain are HLA-DR, HLA-DQ or HLA-DP monomers.

67. The method as claimed in any of claims 55 to 66, where the first chain contains a first functional group and is immobilized on the surface of the nanoparticles by binding

of the first functional group to a second functional group present on the surface of the nanoparticles.

68. The method as claimed in any of claims 55 to 67, where the second chain contains a third functional group and is immobilized on the surface of the nanoparticles by binding of the third functional group to a fourth functional group present on the surface of the nanoparticles.

69. The method as claimed in claim 67, where the first functional group is a natural component of the first chain or is introduced into the first chain by genetic engineering, biochemical, enzymatic and/or chemical derivatization or chemical synthesis.

70. The method as claimed in claim 68, where the third functional group is a natural component of the second chain or is introduced into the second chain by genetic engineering, biochemical, enzymatic and/or chemical derivatization or chemical synthesis.

71. The method as claimed in claim 69 or 70, where the first and the third functional group are different from one another and are selected from the group consisting of carboxyl groups, amino groups, thiol groups, biotin groups, His tag, FLAG tag, Strep tag I groups, Strep tag II groups, histidine tag groups and FLAG tag groups.

72. The method as claimed in any of claims 67 or 68, where the second and fourth functional groups present on the surface of the nanoparticles are different from one another

and are selected from the group consisting of amino groups, carboxyl groups, maleinimido groups, avidin groups, streptavidin groups, neutravidin groups and metal chelate complexes.

73. The method as claimed in claim 72, where the second and the fourth functional group are applied to the surface of the nanoparticles by graft silanization, silanization, chemical derivatization and similar suitable processes.

74. The method as claimed in claim 72 or 73, where the nanoparticles have a core of a chemically inert material, preferably silicon dioxide, and a diameter of from 30 to 400 nm, preferably from 50 to 150 nm.

75. The method as claimed in any of claims 55 to 74, where the nanoparticles which have a first immobilized chain on their surface are obtained by the following steps:

- a) incubation of the first chain which contains the first functional group, of the second chain and of a peptide whose amino acid sequence is known and which is known to be capable of forming a peptide-presenting MHC molecule under suitable conditions,
- b) incubation of the peptide-presenting MHC molecule with nanoparticles whose surface has at least one second functional group which binds the first functional group, under conditions suitable for immobilizing the peptide-presenting MHC molecule on the nanoparticles,

- c) treatment of the nanoparticles having the immobilized peptide-presenting MHC molecules with a suitable buffer to remove the second chain and the peptide having a known amino acid sequence from the immobilized MHC molecule, and
- d) purification of the nanoparticles having the first immobilized chain.

76. The method as claimed in any of claims 55 to 75, where the competitive binding of the peptide fragment population to the nanoparticles having the first immobilized chain is carried out by incubating the peptide fragment population with the nanoparticles in a suitable buffer under suitable conditions.

77. The method as claimed in any of claims 55 to 76, where the nanoparticles are, after binding of the peptide fragment having the highest affinity of the population and the second chain with formation of an immobilized peptide fragment-presenting MHC molecule, removed by centrifugation and washing from the buffer and the unbound peptide fragments.

78. The method as claimed in any of claims 55 to 77, where the nanoparticles having the immobilized peptide fragment-presenting MHC molecule are treated with a buffer suitable for releasing the bound peptide fragment.

79. The method as claimed in any of claims 55 to 78, where the released peptide fragment is isolated and its amino

acid sequence is determined.

80. The method as claimed in any of claims 55 to 79, where, based on the determined amino acid sequence of the released peptide fragment, a nucleic acid coding for the determined amino acid sequence is generated and inserted into a suitable expression vector, which vector containing the nucleic acid is transferred into a host cell suitable for expressing the amino acid sequence and a peptide having the amino acid sequence of the peptide fragment is expressed in the host cell, preferably in a relatively large amount, and isolated therefrom.

81. The method as claimed in any of claims 55 to 79, where, based on the determined amino acid sequence of the released peptide fragment, a suitable amount of a peptide having the amino acid sequence of the peptide fragment is synthesized chemically.

82. A method for controlling the quality of receptor/ligand complexes and/or components thereof, which comprises preparing or providing a receptor/ligand complex in solution of two receptor units, where at least one receptor unit has a first functional group, and a ligand, immobilizing the receptor/ligand complexes on nanoparticles which have, on their surface, at least one second functional group which binds the first functional group, and analyzing the nanoparticles having the immobilized receptor/ligand complex using a MALDI method.

83. The method as claimed in claim 82, where the receptor is an MHC molecule, the ligand is a peptide of known sequence and defined length which binds to the receptor and the receptor/ligand complex is a peptide-presenting MHC molecule.

84. The method as claimed in claim 82 or 83, where the receptor is an MHC molecule of class I, one receptor unit is a heavy chain of about 45 kDa and one receptor unit is a light chain of about 12 kDa.

85. The method as claimed in claim 84, where the heavy chain is an HLA-A, HLA-B or HLA-C monomer and the light chain is β -2-microglobulin.

86. The method as claimed in claim 82 or 83, where the receptor is an MHC molecule of class II, one receptor unit is an α -chain of about 34 kDa and one receptor unit is a β -chain of about 30 kDa.

87. The method as claimed in claim 86, where the α -chain and the β -chain are HLA-DR, HLA-DQ or HLA-DP monomers.

88. The method as claimed in any of claims 82 to 87, where the MALDI method is a MALDI-TOF method.

89. A method for preparing nanoparticles having, on their surface, at least one immobilized receptor unit or one immobilized receptor, which comprises

- a) preparing a receptor/ligand complex by incubation of a first receptor unit having a first functional group, a second receptor unit capable of forming, with the first receptor unit, a receptor, and a

ligand in solution,

- b) immobilizing the receptor/ligand complex formed on nanoparticles having, on the surface, at least one second functional group which binds the first functional group, and
- c) treating the nanoparticles having the immobilized receptor/ligand complex with an acidic buffer to release at least the bound ligand, giving nanoparticles having immobilized receptor units.

90. The method as claimed in claim 89, where the immobilization of the receptor/ligand complex on the nanoparticle surface is only carried out via the first functional group of the first receptor unit binding to the second functional group of the nanoparticles.

91. The method as claimed in claim 89 or 90, where after the treatment, with an acidic buffer, of the nanoparticles having the immobilized receptor/ligand complex in addition to the ligand the second receptor unit, too, is released, giving nanoparticles having the immobilized first receptor unit.

92. The method as claimed in claim 89, where the second receptor unit has a third functional group and the nanoparticles have, on their surface, a fourth functional group which binds the third functional group of the second receptor unit, so that the immobilization of the receptor/ligand complex on the nanoparticles is via binding of the first functional group of the first receptor unit to

the second functional group of the nanoparticles and binding of the third functional group of the second receptor unit to the fourth functional group of the nanoparticles.

93. The method as claimed in claim 92, where after the treatment of the nanoparticles having the immobilized receptor/ligand complex with an acidic buffer only the ligand is released, giving nanoparticles having immobilized first and second receptor units.

94. The method as claimed in claim 92 or 93, where the first and second receptor units are immobilized in a targeted manner, forming a receptor capable of binding a ligand.

95. The method as claimed in claims 89 to 94, where the receptor is an MHC molecule, the ligand is a peptide of known sequence and defined length which binds to the receptor and the receptor/ligand complex is a peptide-presenting MHC molecule.

96. A method as claimed in claim 95, where the receptor is an MHC molecule of class I.

97. The method as claimed in claim 95 or 96, where the first receptor unit is a heavy chain of about 45 kDa and the second receptor unit is a light chain of about 12 kDa or the first receptor unit is a light chain of about 12 kDa and the second receptor unit is a heavy chain of about 45 kDa.

98. The method as claimed in claim 97, where the heavy chain is an HLA-A, HLA-B or HLA-C monomer and the light chain is β -2-microglobulin.

99. The method as claimed in claim 95, where the receptor is an MHC molecule of class II.

100. The method as claimed in claim 99, where the first receptor unit is an α -chain of about 34 kDa and the second receptor unit is a β -chain of about 30 kDa or the first receptor unit is a β -chain of about 30 kDa and the second receptor unit is an α -chain of about 34 kDa.

101. The method as claimed in claim 100, where the α -chain and the β -chain are HLA-DR, HLA-DQ or HLA-DP monomers.

102. The method as claimed in any of claims 89 to 101, where the first functional group and the third functional group are different from one another and are selected from the group consisting of carboxyl groups, amino groups, thiol groups, biotin groups, His tag, FLAG tag, Strep tag I groups, Strep tag II groups, histidine tag groups and FLAG tag groups.

103. The method as claimed in any of claims 89 to 102, where the second functional group on the surface of the nanoparticle, which binds the first functional group, and the fourth functional group on the surface of the nanoparticle, which binds the third functional group, are different from one another and selected from the group consisting of amino groups, carboxyl groups, maleinimido groups, avidin groups, streptavidin groups, neutravidin groups and metal chelate complexes.

104. The method as claimed in any of claims 89 to 103, where the nanoparticles which have the immobilized receptor/peptide complex are treated with a stripping buffer, pH 3.0, which comprises 50 mM sodium citrate, for a period of less than 20 s, preferably 10 s, to remove the bound peptide.

105. A method for preparing nanoparticles having immobilized peptide-presenting MHC molecules, where nanoparticles having at least one first immobilized chain of an MHC molecule preparable by a method according to any of claims 89 to 104 are incubated in the presence of a second chain capable of forming an MHC molecule with the first chain, with a peptide capable of binding to the MHC molecule, giving a peptide-presenting MHC molecule immobilized on the nanoparticles.

106. The method as claimed in claim 105, where the MHC molecule is a molecule of class I and the peptide has a length of about 8 to about 10 amino acids.

107. The method as claimed in claim 105, where the MHC molecule is a molecule of class II and the peptide has a length of about 15 to about 24 amino acids.

108. A method for enriching and/or isolating specific CD4⁺-T-lymphocytes or CD8⁺-T-lymphocytes from peripheral blood mononuclear cells (PBMCs), which comprises

- a) preparing nanoparticles having immobilized peptide-presenting MHC molecules as claimed in any of claims 105 to 107, where the peptide is a T-cell

epitope,

- b) isolating peripheral blood mononuclear cells from a suitable starting material,
- c) incubating the isolated blood mononuclear cells with the nanoparticles having the immobilized peptide-presenting MHC molecules, the T-lymphocytes binding to the T-cell epitope of the immobilized peptide-presenting MHC molecules,
- d) removing the nanoparticles having the T-lymphocytes bound to the immobilized peptide-presenting MHC molecules from the unbound peripheral mononuclear cells.

109. The method as claimed in claim 108, where the bound T-lymphocytes are released from the nanoparticles.

110. The method as claimed in claim 109, where the released T-lymphocytes are propagated by in vitro cloning.

111. The method as claimed in claim 109 or 110, where the released and/or clonally propagated T-lymphocytes are introduced into an organism.

112. The method as claimed in any of claims 108 to 111, where the peptide-presenting MHC molecule is a molecule of class I and the bound T-lymphocytes are CD8⁺-T-lymphocytes.

113. The method as claimed in any of claims 108 to 111, where the peptide-presenting MHC molecule is a molecule of class II and the bound T-lymphocytes are CD4⁺-T-lymphocytes.

114. A method for priming and/or restimulating a $CD4^{+}$ -T- or $CD8^{+}$ -T-lymphocyte reaction in vitro, which comprises

- a) identifying a T-cell epitope as claimed in any of claims 1 to 54 and determining its amino acid sequence,
- b) preparing a nucleic acid coding for a peptide having the amino acid sequence of the T-cell epitope,
- c) introducing the nucleic acid prepared under b) into a suitable vector,
- d) introducing the vector obtained under c) into dendritic cells isolated, if appropriate, from cultivated peripheral blood mononuclear cells,
- e) propagating the dendritic cells obtained under d), which have the vector, in vitro, and
- f) stimulating autologous $CD4^{+}$ - and/or $CD8^{+}$ -cells in vitro using the dendritic cells obtained under d) or e).

115. A nanoparticle, comprising on the surface at least one receptor unit, in particular an immobilized chain of an MHC molecule.

116. The nanoparticle as claimed in claim 115, where the immobilized chain may, by binding a peptide of 8 to 24 amino acids and a second chain of an MHC molecule, form a peptide-presenting MHC molecule.

117. The nanoparticle as claimed in claim 115 or 116, where the MHC molecule chain is immobilized on the nanoparticle surface by binding of a first functional group present in the chain to a second functional group present on the nanoparticle surface.

118. The nanoparticle as claimed in any of claims 115 to 117, where the MHC molecule is a molecule of class I and consists of a heavy chain of about 45 kDa and a light chain of about 12 kDa.

119. The nanoparticle as claimed in claim 118, where either the heavy chain or the light chain is immobilized.

120. The nanoparticle as claimed in any of claims 115 to 117, where the MHC molecule is a molecule of class II and consists of an α -chain of about 34 kDa and a β -chain of about 30 kDa.

121. The nanoparticle as claimed in claim 120, where either the α -chain or the β -chain is immobilized.

122. A nanoparticle having an immobilized MHC molecule, where the MHC molecule comprises a first and a second chain and the MHC molecule is immobilized on the nanoparticle surface by binding of a first functional group present in the first chain to a second functional group present on the nanoparticle surface or by binding of the first functional group present in the first chain to the second functional group present on the nanoparticle surface and binding of a third functional group present in the second chain to a

fourth functional group present on the nanoparticle surface.

123. A nanoparticle having a peptide-presenting MHC molecule immobilized on the nanoparticle surface, where the peptide-presenting MHC molecule comprises a first chain, a second chain and a peptide of 8 to 24 amino acids and the MHC molecule is immobilized on the nanoparticle surface by binding of a first functional group present in the first chain to a second functional group present on the nanoparticle surface or by binding of the first functional group present in the first chain to the second functional group present on the nanoparticle surface and binding of a third functional group present in the second chain to a fourth functional group present on the nanoparticle surface.

124. The nanoparticle as claimed in claim 122 or 123, where the MHC molecule is a molecule of class I and consists of a heavy chain of about 45 kDa and a light chain of about 12 kDa.

125. The nanoparticle as claimed in claim 124, where the first chain is the heavy chain and the second chain is the light chain or where the first chain is the light chain and the second chain is the heavy chain.

126. The nanoparticle as claimed in claim 122 or 123, where the MHC molecule is a molecule of class II and consists of an α -chain of about 34 kDa and a β -chain of about 30 kDa.

127. The nanoparticle as claimed in claim 126, where the first chain is the α -chain and the second chain is the β -

chain or where the first chain is the β -chain and the second chain is the α -chain.

128. A peptide vaccine which comprises at least one peptide-presenting MHC molecule preparable as claimed in any of claims 55 to 81 and/or which comprises at least one protein antigen which contains a T-cell epitope identifiable by the methods as claimed in claims 1 to 54.

129. The peptide vaccine as claimed in claim 128, where the vaccine is present as a lyophilizate.

130. The peptide vaccine as claimed in claim 128, where the vaccine is present as an aqueous colloidal solution or suspension.

131. The peptide vaccine as claimed in any of claims 128 to 130, additionally comprising at least one adjuvant.

132. A kit for identifying and/or detecting T-cell epitopes of a protein antigen in vitro, comprising a container with a suspension of nanoparticles having an immobilized MHC molecule as claimed in any of claims 122 to 127 or a container with a suspension of nanoparticles having an immobilized first chain of an MHC molecule as claimed in any of claims 115 to 121 and a container with a lyophilizate of a second chain.

133. The use of a nanoparticle as claimed in any of claims 115 to 127 for identifying and/or for detecting T-cell epitopes of a protein antigen in vitro.

134. The use of a nanoparticle as claimed in any of claims 115 to 127 for preparing a peptide vaccine.

135. The use of a nanoparticle as claimed in any of claims 115 to 127 for enriching and/or isolating specific CD4⁺-T-lymphocytes or CD8⁺-T-lymphocytes in vitro.

136. The use of a nanoparticle as claimed in any of claims 115 to 127 for priming and/or restimulating a CD4⁺- and/or CD8⁺-T-lymphocyte reaction in vitro.

137. The use of a peptide vaccine as claimed in any of claims 128 to 131 for the active immunization of an animal or human organism against a protein antigen.